Pollen Grain Counting Using a Cell Counter

Kakui, Hiroyuki ; Yamazaki, Misako ; Hamaya, Naoto-Benjamin ; Shimizu, Kentaro K

Abstract: The number of pollen grains is a critical part of the reproductive strategies in plants and varies greatly between and within species. In agriculture, pollen viability is important for crop breeding. It is a laborious work to count pollen tubes using a counting chamber under a microscope. Here, we present a method of counting the number of pollen grains using a cell counter. In this method, the counting step is shortened to 3 min per flower, which, in our setting, is more than five times faster than the counting chamber method. This technique is applicable to species with a lower and higher number of pollen grains, as it can count particles in a wide range, from 0 to 20,000 particles, in one measurement. The cell counter also estimates the size of the particles together with the number. Because aborted pollen shows abnormal membrane characteristics and/or a distorted or smaller shape, a cell counter can quantify the number of normal and aborted pollen separately. We explain how to count the number of pollen grains and measure pollen size in Arabidopsis thaliana, Arabidopsis kamchatica, and wheat (Triticum aestivum).

DOI: https://doi.org/10.1007/978-1-0716-0672-8_1

Fig. 1

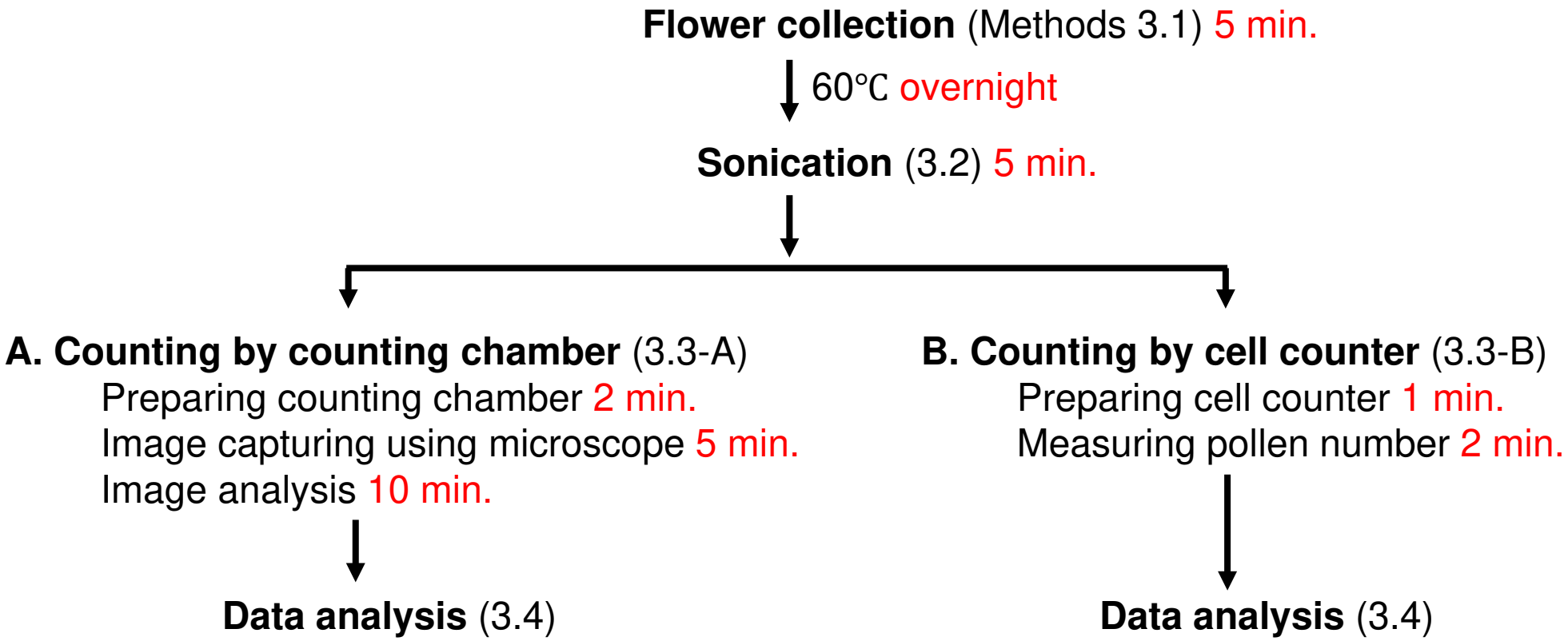

Flower collection (Methods 3.1) 5 min.
60℃ overnight
Sonication (3.2) 5 min.
A. Counting by counting chamber (3.3-A)
Preparing counting chamber 2 min.
Image capturing using microscope 5 min.
Image analysis 10 min.
Data analysis (3.4)
B. Counting by cell counter (3.3-B)
Preparing cell counter 1 min.
Measuring pollen number 2 min.
Data analysis (3.4)

Fig. 2

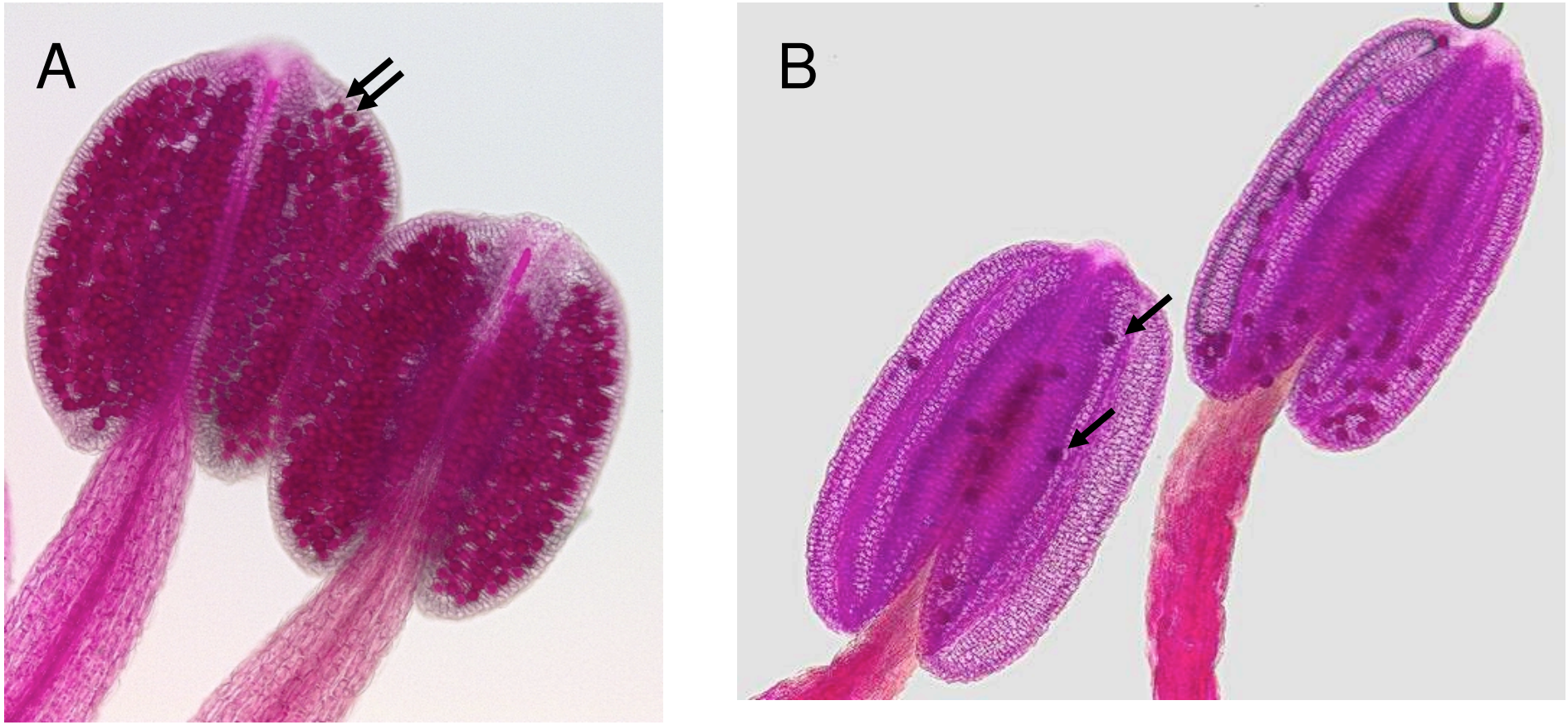


Fig. 3

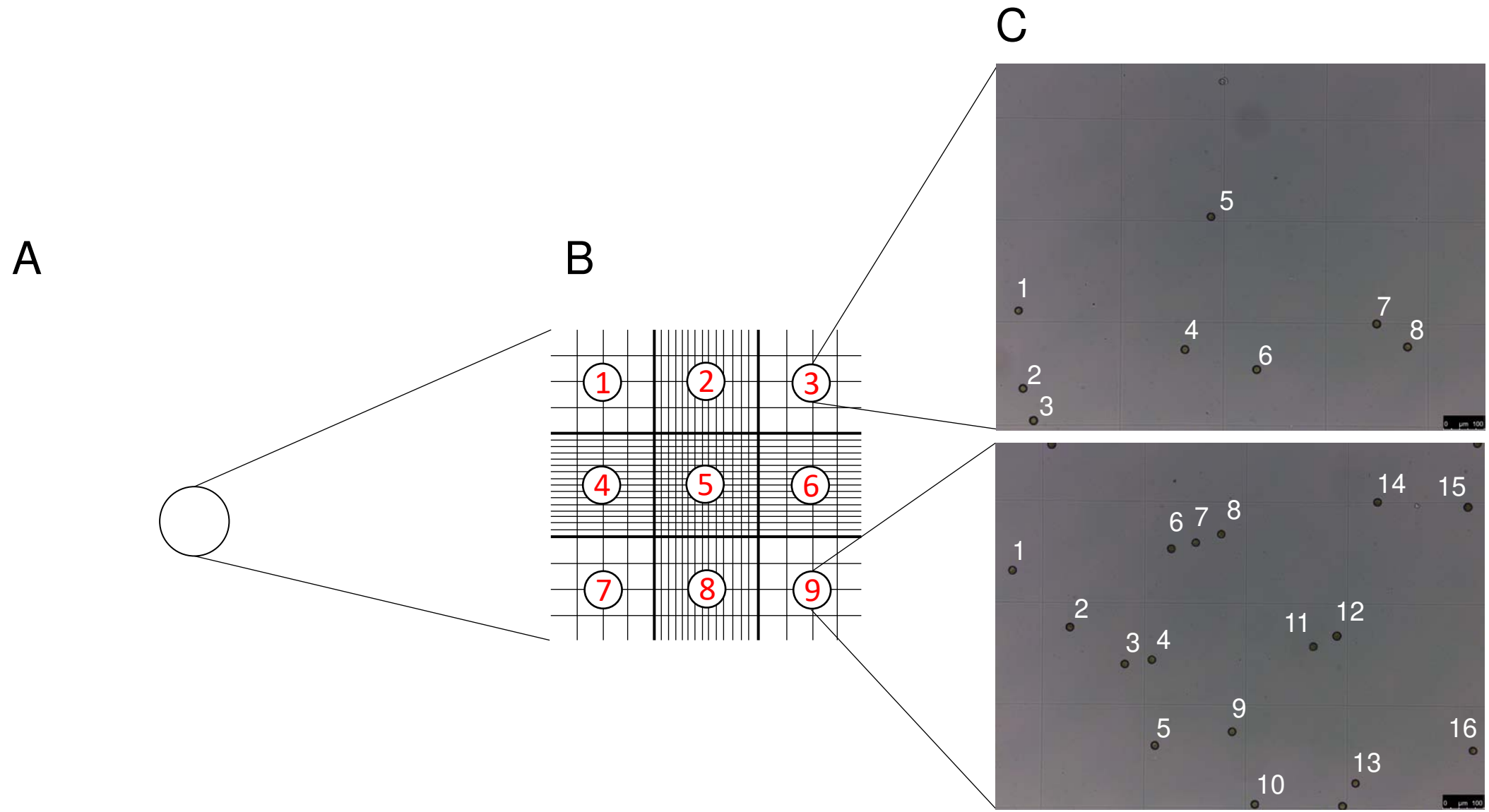
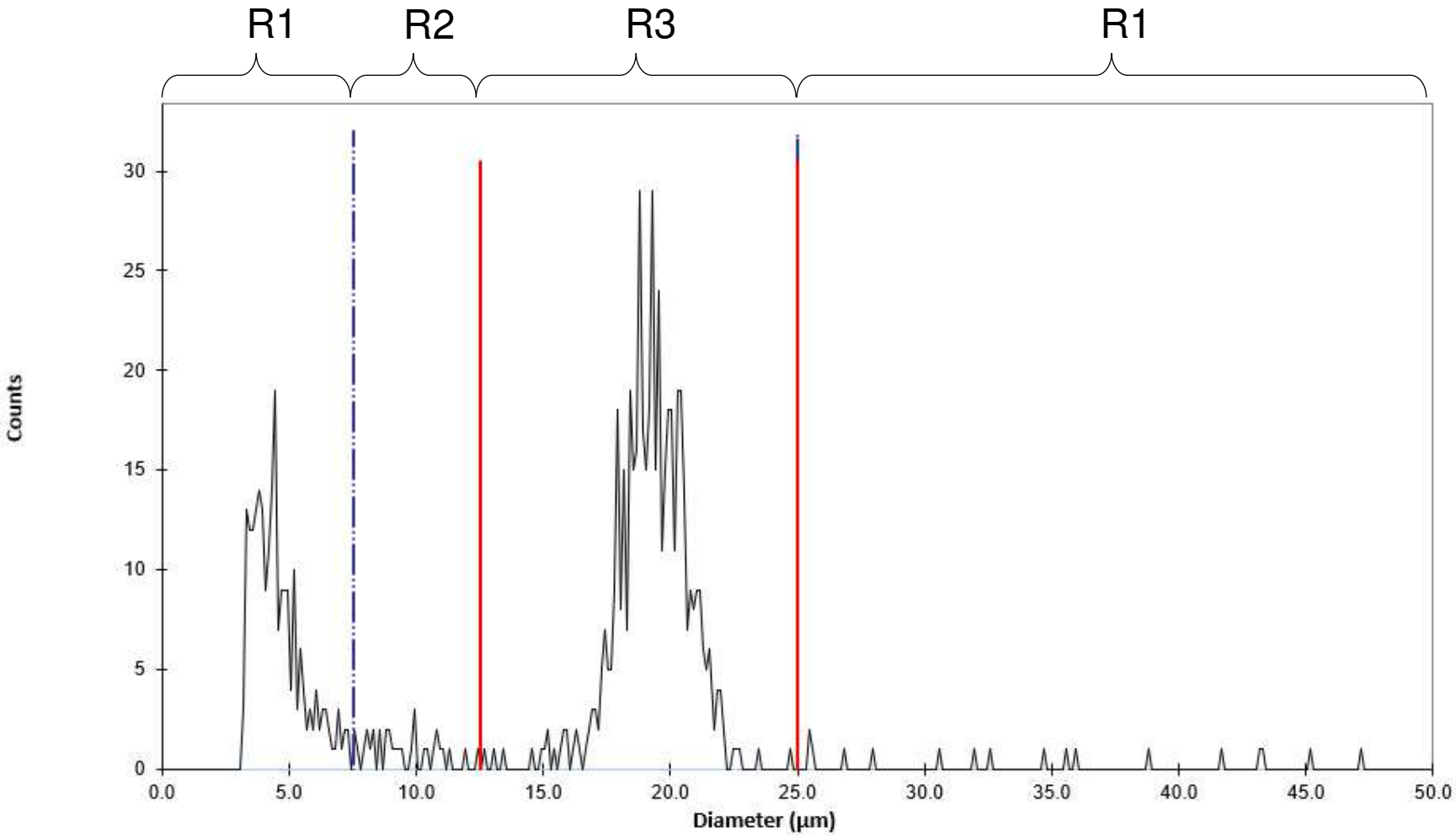
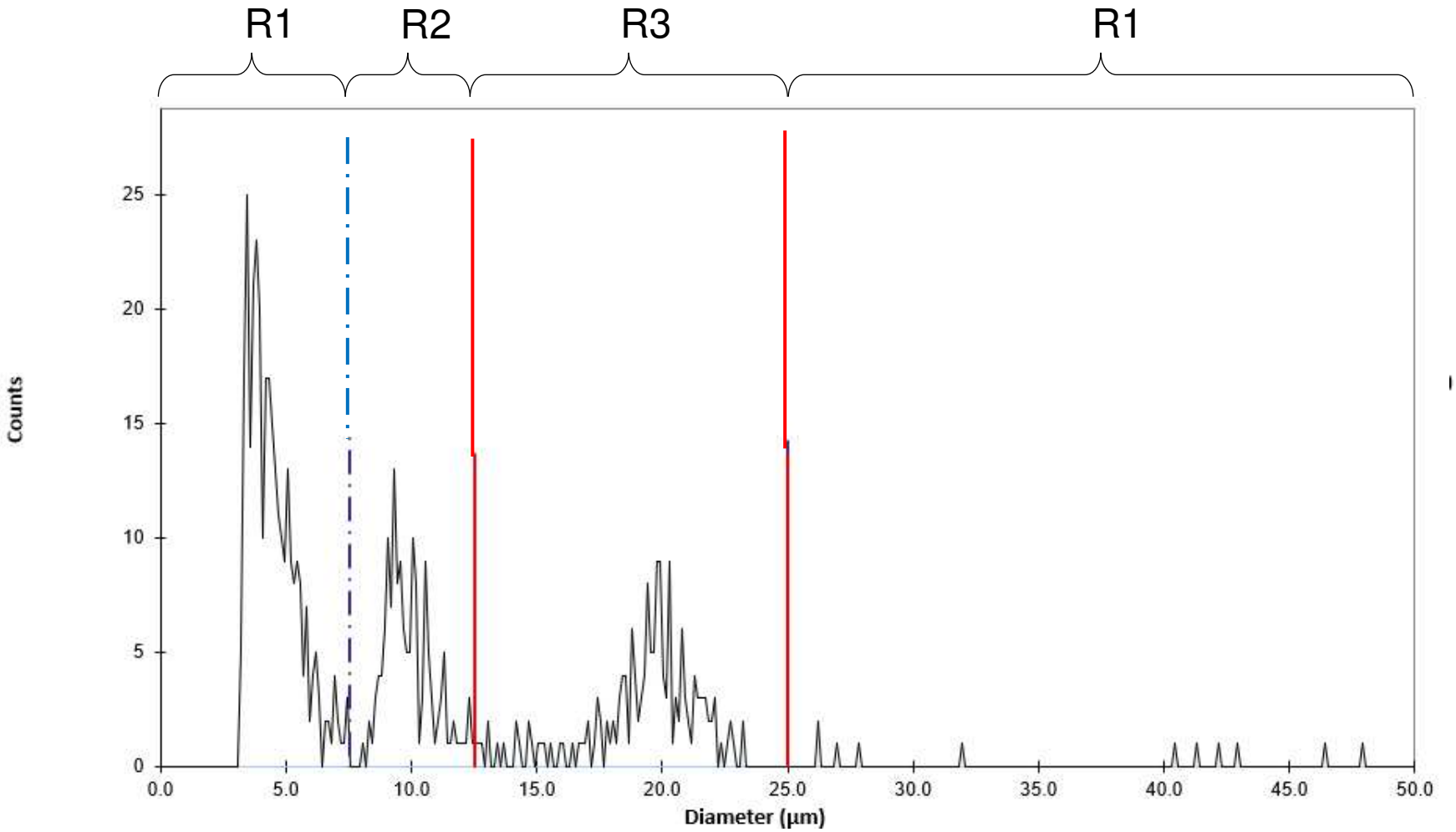


Fig. 4A



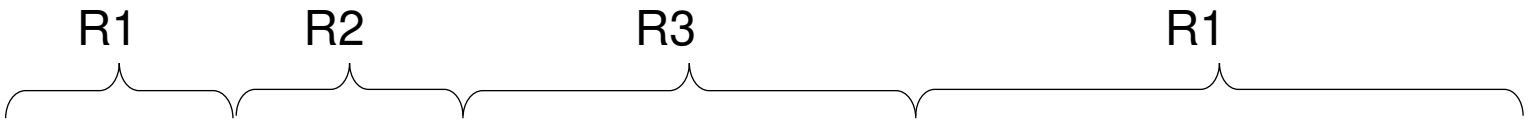
Results	*modified*
Conc. check	Ok
Counts	506
Counts/ml > 50 µm	8.692E+00
Aggr.factor	1.000
Viable cells/ml	4.398E+03
Total cells/ml	4.684E+03
%Viability	93.9 %
Debris/ml	1.955E+03
Volume/ml	1.680E+07 fl
Mean volume	3.821E+03 fl
Peak volume	3.770E+03 fl
Mean diameter	19.29 µm
Peak diameter	19.31 µm

Fig. 4B



Results	*modified*
Conc. check	Ok
Counts	165
Counts/ml > 50 µm	0.000E+00
Aggr.factor	1.000
Viable cells/ml	1.434E+03
Total cells/ml	2.703E+03
%Viability	53.1 %
Debris/ml	2.738E+03
Volume/ml	5.526E+06 fl
Mean volume	3.853E+03 fl
Peak volume	4.079E+03 fl
Mean diameter	19.20 µm
Peak diameter	19.82 µm

Fig. 4C



Results	*unchanged*
Conc. check	Ok
Counts	1997
Counts/ml > 50 µm	3.477E+01
Aggr.factor	1.000
Viable cells/ml	1.736E+04
Total cells/ml	1.853E+04
%Viability	93.7 %
Debris/ml	5.041E+03
Volume/ml	8.659E+07 fl
Mean volume	4.989E+03 fl
Peak volume	4.440E+03 fl
Mean diameter	21.09 µm
Peak diameter	20.39 µm

Fig. 4D

Results	*unchanged*
Conc. check	Ok
Counts	205
Counts/ml > 80 µm	1.043E+02
Aggr.factor	1.000
Viable cells/ml	1.782E+03
Total cells/ml	2.095E+03
%Viability	85.1 %
Debris/ml	8.640E+03
Volume/ml	1.084E+08 fl
Mean volume	6.084E+04 fl
Peak volume	6.043E+04 fl
Mean diameter	48.61 µm
Peak diameter	48.69 µm

Fig. 5

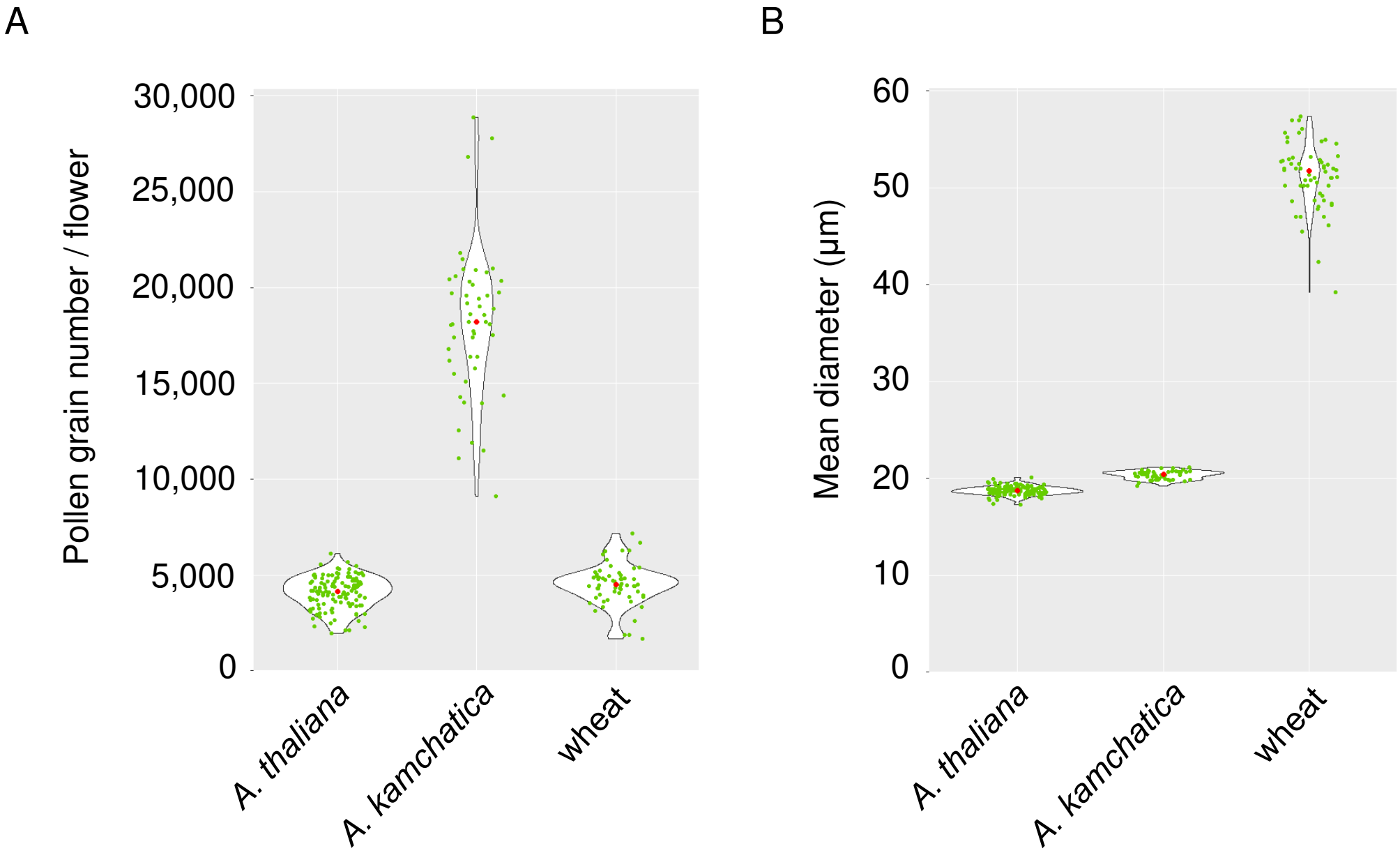

A
Pollen grain number / flower
30,000
25,000
20,000
15,000
10,000
5,000
0
A. thaliana
A. kamchatica
wheat
B
Mean diameter (μm)
60
50
40
30
20
10
0
A. thaliana
A. kamchatica
wheat

Fig. 6